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Today, fourteen years after the discovery of the B19 virus, the diagnostics for infection with the virus are still performed in only a limited number of laboratories in the world. Because the virus cannot be demonstrated anymore in the
5   patients at the time when the symptoms arise (viremia and virus excretion precede the symptoms), diagnostics must focus on demonstrating B19-specific (IgM)-antibodies.

To this end (and also for the preparation of suitable vaccines, for example) it is necessary to have a sufficient
10   supply of B19-antigen for setting up the tests. What is lacking, however, is a suitable *in vitro* cell culture system for propagating the virus, with which sufficient antigen can be obtained.

The existing parvovirus B19 diagnostics are performed
15   with virus antigen which becomes available more or less by chance (screening blood donors offers an estimated chance of 1 in 50,000 that viremic blood is found).

For these reasons there is a great need for antigen which is produced using recombinant DNA techniques. Accordingly,
20   various proposals in this direction have already been made, but none of them has proved really useful for the construction of a diagnostic test.

The present invention is based on the use of an expression vector system that was developed fairly recently,
25   viz. the "Baculovirus Expression Vector System". In this system use is made of a recombinant virus vector of the baculo-virus *Autographica californica* nuclear polyhedrosis virus (AcNPV) to express the B19 virus proteins in insect cells: *Spodoptera frugiperda* (Sf9). This system offers many
30   advantages over the current systems of expression vectors:
a)   In view of the use for diagnostic and possibly therapeutic (vaccination) purposes, no cross reactivity is to be expected against proteins of the baculovirus or the insect cells (in proteins which are expressed in
35   E.coli, this cannot always be precluded).

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b)   The virus proteins can be produced in large amounts
     (1-500 mg/l) up to even 50-75% of the total protein,
     detected on SDS-polyacrylamide gel (Summers and Smith,
     1986, a manual of methods for baculovirus vector and
5   insect cell culture procedures; Yong Kang, 1988; Adv. in
     Virus Res. 35, 177-192). These are considerably larger
     amounts than those produced in prokaryotic expression
     systems or in Chinese hamster ovary cells, as described
     by Kajigaya et al. (Blood 75(5), suppl.1, 44a, abstr. 86;
10   1988).

c)   The proteins can be produced as non-fusion proteins, in
     contrast to for instance the B19 protein, which has been
     produced as a fusion protein in E.coli by Sisk and Berman
     (Biotechnology 5, 1077-1080, 1987). This recombinant
15   β-galactosidase-B19 fusion protein goes into solution
     only in the presence of sodium dodecylsulphate (SDS). The
     proteins VP1 and VP2 expressed in insect cells in
     accordance with the invention, by contrast, can easily be
     dissolved by sonification of the cells in a buffer which
20   contains 25 mM $NaHCO_3$ and 20 mg/l NaN_3 (pH 9.5). In such a
     treatment 95% of the cellular proteins go over into the
     soluble supernatant fraction.

d)   The proteins can be produced in an insect cell line which
     is easy to culture, as opposed to the production of virus
25   proteins in human erythroid bone marrow cells (Ozawa et
     al., 1987; Blood 70, 384-391) or human foetal erythroid
     liver cells (Yaegashi et al., 1989; J. Virol. 63,
     2422-2426).

e)   Because in the baculovirus expression vector system pre-
30   and post-translation modifications occur, such as
     phosphorylation, glycosylation, signal peptide split-off
     and the removal of introns by splicing, the system is
     potentially very suitable for the production of
     biologically active proteins with a (virtually) native
35   structure (Yong Kang, 1988; Adv. in Virus Res. 35,
     177-192). In this system VP1 and VP2 of B19 can be

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expressed both separately and collectively. Moreover, the
possibility exists that virus-like particles are
spontaneously formed from one or more of these proteins.

f)   An additional advantage of the baculovirus is that it
5   does not multiply in mammalian cells and hence is not
        pathogenic for humans, which makes it much safer to work
        with and utilize this system.

        According to the invention it has actually been
10   accomplished to produce in a high yield the coat proteins VP1
     and VP2 of the human parvovirus B19 in an antigenically active
     form as non-fusion proteins, as virus-like particles or not,
     using the baculovirus expression system in insect cells
     (Spodoptera frugiperda). Further, it has been accomplished to
15   develop, using the B19 virus proteins producing insect cells,
     a specific and sensitive immunofluorescence-assay (IFA) and a
     specific and sensitive Enzyme-Linked-Immuno-Sorbent-Assay
     (ELISA) for the detection of antibodies directed against the
     B19 virus proteins. However, on the basis of the B19 virus
20   proteins and virus-like particles produced in insect cells in
     conformity with the invention, other diagnostic assays can be
     developed as well, such as a Radio-Immuno-Assay (RIA) or an
     agglutination test.

        The invention is primarily embodied in recombinant VP1
25   and VP2 protein of the human parvovirus B19, formed in
     Spodoptera frugiperda cells which, by means of a baculovirus
     expression vector system, have been equipped with the genetic
     information necessary for expression of the B19 virus protein
     VP1 and/or VP2. The invention further comprises recombinant
30   virus-like particles which consist of VP2 protein or of VP1
     and VP2 protein of the human parvovirus B19, formed in
     Spodoptera frugiperda cells which, by means of a baculovirus
     expression vector system, have been equipped with the genetic
     information necessary for expression of VP2 protein or of VP1
35   and VP2 protein.

Further, the invention is embodied in _Spodoptera
frugiperda_ cells which, by means of a baculovirus expression
vector system, have been equipped with the genetic information
which is necessary for expression of VP1 and/or VP2 protein of
5   the human parvovirus B19.

The invention further provides a method of producing VP1
and/or VP2 protein of the human parvovirus B19 (optionally in
the form of virus-like particles which are composed of VP2
protein or of both proteins) by culturing _Spodoptera
10 frugiperda_ cells which, by means of a baculovirus expression
vector system, have been equipped with the genetic information
which is necessary for expression of the B19 virus protein or
the B19 virus proteins. Optionally and preferably the B19
virus protein formed in the cells and/or the virus-like
15 particles formed in the cells and consisting of VP2 protein or
of VP1 _and_ VP2 protein are isolated from the cells. A suitable
method for that purpose comprises a sonification of the cells
in a buffer which contains 25 mM $NaHCO_3$ and 20 mg/l NaN_3 (pH
9.5). The result of such a treatment is that a great part of
20 the proteins present in the cells, for instance 95%, are
obtained in dissolved form in the supernatant. By known per se
purification methods, the B19 virus proteins can be isolated
at a higher purity.

The invention is also embodied in recombinant baculovirus
25 expression vectors, equipped with the genetic information
which is necessary for expression of VP1 and/or VP2 protein of
the human parvovirus B19 in _Spodoptera frugiperda_ cells.
Preferred embodiments of such recombinant baculovirus
expression vectors are the plasmids pAcB19VP1-YM1 and
30 pAcB19VP2-YM1, to be described hereinafter.

The invention is further embodied in recombinant
baculoviruses, equipped with the genetic information which is
necessary for expression of VP1 and/or VP2 protein of the
human parvovirus B19 in _Spodoptera frugiperda_ cells. Preferred
35 embodiments of such recombinant baculoviruses are the viruses
AcB19VP1L and AcB19VP2L, to be described hereinafter.

The invention further comprises the use of recombinant VP1 and/or VP2 protein of the human parvovirus B19, formed in _Spodoptera frugiperda_ cells which, by means of a baculovirus expression vector system, have been equipped with the genetic
5 information necessary for expression of the B19 virus protein, in an assay for detecting antibodies directed against the B19 virus protein in a sample to be tested. The invention comprises the use of recombinant virus-like particles which consist of VP2 protein or of VP1 _and_ VP2 protein of the human
10 parvovirus B19, formed in _Spodoptera frugiperda_ cells which, by means of a baculovirus expression vector system, have been equipped with the genetic information necessary for expression of these B19 virus proteins, in an assay for detecting antibodies directed against the B19 virus in a sample to be
15 tested. In preferred embodiments of the invention, this concerns the use of _Spodoptera frugiperda_ cells which, by means of a baculovirus expression vector system, have been equipped with the genetic information which is necessary for expression of VP1 and/or VP2 protein of the human parvovirus
20 B19, in an assay for detecting antibodies directed against the B19 virus protein in a sample to be tested, more particularly in an IFA or ELISA for detecting antibodies directed against the B19 virus protein in a sample to be tested.

The invention also comprises a vaccine preparation for
25 inducing an immune response which provides protection against the human parvovirus B19, comprising recombinant VP1 and/or VP2 protein of the human parvovirus B19, formed in _Spodoptera frugiperda_ cells which, by means of a baculovirus expression vector system, have been equipped with the genetic information
30 necessary for expression of the B19 virus protein, or an antigenically active portion of this recombinant B19 virus protein, in combination with one or more carriers and/or adjuvants suitable for vaccination purposes, and further, a vaccine preparation for inducing an immune response which
35 provides protection against the human parvovirus B19, comprising recombinant virus-like particles which consist of

VP2 protein or of VP1 _and_ VP2 protein of the human parvovirus
B19, formed in Spodoptera frugiperda cells which, by means of
a baculovirus expression vector system, have been equipped
with the genetic information necessary for expression of these
5   B19 virus proteins, in combination with one or more carriers
and/or adjuvants suitable for vaccination purposes.

     The invention further comprises the use of recombinant
VP1 and/or VP2 protein of the human parvovirus B19 (or
virus-like particles consisting of VP2 or of VP1 _and_ VP2),
10   formed in Spodoptera frugiperda cells which, by means of a
baculovirus expression vector system, have been equipped with
the genetic information necessary for expression of the B19
virus protein, or with an antigenically active portion of this
recombinant B19 virus protein, for inducing an immune response
15   which provides protection against the human parvovirus B19.

     The invention also comprises the use of virus-like
particles consisting of VP2 protein or VP1 and VP2 protein of
the human parvovirus B19, into which one or more epitopes of
proteins of other pathogens have been incorporated, for
20   inducing an immune response which provides protection against
these other pathogens.

     In the experimental section to follow hereinbelow, it is
shown by way of explanation and illustration how the invention
was carried out and can be carried out. As shown by the
25   Examples, the DNA sequences coding for the structural proteins
VP1 and VP2 of the human parvovirus B19 were isolated from the
B19 virus from the serum of a patient. Then, via subcloning
steps in pUC19 and pUC7, the B19-DNA was cloned into the
baculovirus vector pAcYM1 behind the promoter for the
30   polyhedrin gene of the baculovirus. By means of cotransfection
of this recombinant vector with wild type baculovirus DNA,
followed by recombination in the insect cells (Spodoptera
frugiperda), finally, recombinant virus was isolated which,
after infection in the insect cells, led to the production of
35   the coat proteins VP1 and VP2 of B19, which were or were not
in the form of virus-like particles. Using these B19 proteins,

sensitive and specific IFA and ELISA tests were developed,
enabling fast and simple detection of B19-specific antibodies.
The proteins produced in this manner, which may or may not be
in the form of virus-like particles, may also serve as easily
5 obtainable antigens for other diagnostic tests, such as RIA's
and agglutination tests and for the (possible) production of
vaccines and subunit vaccines.

Description of the Figures
10   Fig. 1 shows the genetic structure of the human
parvovirus B19, which is a single-stranded DNA virus having a
DNA of about 5500 nucleotides. According to Ozawa et al.,
1987; J. Virol. 61, 2395-2406 the nucleotides 2444-4787
contain the sequence coding for VP1 (84 kd) and the
15 nucleotides 3125-4787 contain the sequence coding for VP2 (58
kd). Not shown are the 4 splicing donor-sites, located between
the nucleotides 2177 and 2195, and the 2 acceptor-sites,
located between the nucleotides 3043 and 3050. For the
production of VP2 during the virus replication, the
20 intermediate sequence (nucleotides 2177-3050, which includes
the initiation codon for VP1) is removed by splicing.
       Fig. 1 further shows the cloning diagram for the
construction of recombinant baculovirus with human parvorvirus
B19 genes.
25
Example 1: Expression of parvovirus B19 VP1.

1. Isolation of parvovirus B19 DNA from patient serum.
After B19 DNA was demonstrated in the serum of a patient by
30 means of the "polymerase chain reaction" and Dot-Spot-
Hybridization with B19 specific DNA probes (Salimans et al.,
1989; J. Virol. Meth. 23, 19-28) the B19 DNA was isolated from
it by incubation with proteinase K and SDS (1 ml serum was
incubated at 37°C for 2 h with 100 µg proteinase K in a buffer
35 which contained 10 mM Tris-Cl (pH 7.5), 5 mM EDTA and 0.5%
SDS), phenol extraction for removing the protein and DNA

precipitation by means of ethanol. The DNA was assayed as to
size, restriction enzyme patterns and Southern blot analysis
(Maniatis et al., 1982; Molecular cloning: a laboratory
manual. Cold Spring Harbor, N.Y.) with B19-specific DNA
5   probes.

2. Subcloning into pUC19 (Fig. 1).
The techniques for the manipulations with B19-DNA and
plasmid-DNA were essentially carried out as described by
10 Maniatis et al. (1982; Molecular cloning: a laboratory
manual). The B19 DNA was cut with HindIII (bp 2430) and ScaI
(bp 4920) and ligated into pUC19 (cut with HindIII and SmaI)
so as to obtain a larger amount of the coding sequence. (By
ligating ScaI in SmaI, this cutting site was removed). The
15 plasmid obtained (pUC19-B19VP1) was assayed by restriction
enzyme analysis and hybridization and transformed to and
propagated in E.coli JM101.

3. Subcloning into pUC7 (Fig. 1).
20 To create BamHI-sites at the ends of the B19 DNA, the DNA was
cloned into pUC7, which contains a symmetrical polylinker with
two BamHI-sites. The B19 DNA was cut from pUC19 with HindIII
and EcoRI and also with ScaI to remove the residue of the
vector DNA. The B19 DNA fragment with the proper length was
25 isolated from agarose gel, filled in with "Klenow large
fragment polymerase" and ligated with HincII cut pUC7 plasmid
(also blunt-ended). The ligated DNA (PUC7-B19VP1) was
transformed to and propagated in E.coli JM101. Separate
colonies were tested for the presence of B19 DNA by means of
30 restriction enzyme analysis and hybridization with a B19
specific DNA probe (the 700bp PstI-fragment of B19, Salimans
et al., 1989; J. Virol.Meth. 23, 19-28).

4. Cloning into the baculovirus vector pAcYM1 (Fig. 1).
35 The techniques for manipulations with the baculovirus, the
baculovirus vector and the insect cells were essentially

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performed as described by Summers and Smith in 1986 (a manual
of methods for baculovirus vector and insect cell culture
procedures). The restriction site following the polyhedrin
promoter of the baculovirus is a BamHI-site. Since the B19 DNA
5   also contains an internal BamHI-site, the following strategy
was opted for: the B19 DNA was cut from pUC7 with EcoRI and
the remaining part of pUC7 was cut with ScaI (this ScaI
digestion is not necessary for cloning of VP2 DNA). The B19
DNA was isolated from the gel, dephosphorylized with Calf
10   Intestinal Alkaline Phosphatase (CIP) and finally a partial
digestion was performed with BamHI. The BamHI-fragment with
the proper length (2.5 kb for VP1 and 1.8 kb for VP2) was then
ligated with baculovirus vector pAcYM1, cut with BamHI and
treated with CIP (Matsuura et al., 1987; J. Gen. Virol. 68,
15   1233-1250). The plasmid (pAcB19VP1-YM1) thus obtained was
propagated in E. coli HB101. Recombinant plasmids were
analyzed for the presence of B19 DNA (with specific B19 DNA
probes) and the proper orientation thereof relative to the
polyhedrin promoter (by means of restriction enzyme analysis).
20   Constructs with the proper orientation were cultured and
isolated from the bacterial cells according to "the rapid
boiling" method of Holmes and Quigley (1981; Anal.Bioch. 114,
193-197) with a final purification on a CsCl-gradient.

25   5. Cotransfection with wild type baculovirus DNA: AcNPV
(Fig.1).
By means of a cotransfection of the pAcB19VP1-YM1 construct
with wild type baculovirus DNA (AcNPV) the DNA was introduced
into the insect cells (Spodoptera frugiperda-Sf) (according to
30   method II of Summers and Smith, 1986; a manual of methods for
baculovirus vector and insect cell culture procedures). In
0.5-3.0% of the infections, the recombinant vector
(pAcB19VP1-YM1) recombines with the wild type virus DNA during
the uptake into the cells, thus generating a recombinant virus
35   which contains the B19 DNA: AcB19VP1L. The polyhedrin gene has
been replaced by the VP1 DNA (in Example 2, in which the

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formation of a recombinant virus AcB19VP2L is described, by
the VP2 DNA).

6. Purification of recombinant virus (AcB19VP1L and
5   AcB19VP2L).
The titre of the virus (both wild type and recombinant)
produced by the Sf cells was determined by means of a plaque
assay. Twenty plaque-forming units (pfu) were placed on a
monolayer of Sf-cells in a 96-well plate (20 pfu/well) and
10 incubated at 27°C. Three days after the infection the
supernatants were removed and retained and the cells were
lysed and spotted on nitrocellulose (Pet et al., 1989; Nucl.
Acid Res. 17, 451). Screening for the presence of recombinant
virus was performed with a parvovirus B19-specific DNA probe.
15 The titre of the wells containing the collected supernatants
of recombinant virus was determined by means of a plaque assay
and then diluted to 1 pfu/well on a 96-well plate. After a
screening as described hereinabove with the dot-spot-
hybridization assay the titre of the positive supernatants was
20 determined in a plaque assay and furthermore screening for the
absence of the polyhedrin protein in the plaques was done
with a microscope, indicating that the plaque contains
recombinant_ virus. These plaques were inoculated on a Sf-
monolayer in a 96-well plate. After three days dot-spot-
25 hybridization was repeated and positive wells were assayed in
a plaque assay. The recombinants were considered pure when
less than 1 in 500 plaques contained wild type virus.

7. Assay of the recombinant virus (AcB19VP1L).
30 From cells infected with recombinant virus, total DNA was
isolated, cut with restriction enzymes and following Southern
blotting assayed by hybridization with parvovirus B19-specific
DNA probes.
Pure recombinant virus was used to infect insect cells (Sf)
35 with an m.o.i. (multiplicity of infection) of 1-5 and to
express parvovirus VP1 and VP2 in these cells. Then the coat

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proteins VP1 and VP2 of the human parvovirus B19, produced in
the baculovirus expression vector system, were analyzed and
assayed with the techniques described hereinbelow:

5   8. Biosynthesis of recombinant VP1.
Two days after infection with recombinant virus (AcB19VP1L,
m.o.i. 5) insect cells (10^6 Sf-cells in 35 mm-Petri dish) were
cultured for 4 h at 27°C in methionine-free "Grace"-medium to
which 100 uCi ^{35}S-methionine had been added, for determining
10   the de novo synthesis of proteins. After the supernatant was
discarded, the cells were placed in PBS (phosphate buffered
saline). Both supernatant and cell fraction were placed in
lysing buffer, boiled for 5 min and analyzed on a 10% SDS-
polyacrylamide gel. The same experiments were performed with
15   uninfected and wild type baculovirus-infected cells.
Autoradiography (not shown) revealed that the polyhedrin band
(30kd) which dominated strongly in the wild type infection,
disappeared in the recombinant viruses and that a protein of
the size of the VP1 of B19 (84kd) was synthesized.
20

9. Analysis for SDS-PAGE
After infection with recombinant virus (m.o.i.5) Spodoptera
frugiperda cells were analyzed for 5 days for the production
of VP1 of B19 virus on 10% SDS-polyacrylamide gel. The gels
25   were stained with "fast green". The gel results (not shown)
revealed that from day 2 VP1 was produced in large amounts as
a stable product which was still clearly present after 5 days.
VP1 was produced in comparable amounts to those of the
polyhedrin protein in wild type baculovirus-infected cells.
30

10. Antigenicity of the produced proteins.
100 ng of protein of cell lysates of Sf-cells 2 days after
infection (m.o.i.5) was subjected to electrophoresis on 10%
SDS-polyacrylamide gel and blotted to nitrocellulose (3 h; 70V
35   in 25mM Tris, 192 mM glycine and 20% methanol). Then 20 human
sera were tested for reactivity with the recombinant VP1

antigen (15 IgG-positive sera and 5 IgG negative sera). The
reactions were performed at room temperature: overnight
blocking by incubation of the nitrocellulose filter in PBS/ 5%
Foetal Calf Serum (FCS)/ 0.05% Tween 20; incubation with sera
5   (1:500 dilution for the positive sera and 1:50 dilution for
the negative sera) diluted in PBS/ 5% FCS for 1.5 h; followed
by washing of the filters for 1 h with PBS/ 0.05% Tween 20 and
then incubation with 1:500 diluted alkaline phosphatase
labelled goat-anti-human-total Ig (Tago, cat.no.: 2493) in
10 PBS/ 0.05% Tween 20/ 5%FCS; after washing 2x in PBS/ 0.05%
Tween 20 and 2x in PBS, bound antibodies were detected with
5-bromo-4-chloro-3-indolyl phosphate (BCIP). The 15 positive
IgG sera all reacted with the recombinant VP1 band on the gel
and the 5 negative IgG sera did not yield any reaction.
15

11. Testing sera in an immunofluorescence assay.
Sf-cells infected with recombinant virus (AcB19VP1L, m.o.i.1)
were placed in TC100 medium (Gibco-BRL) after 3-4 days, mixed
with uninfected cells up to a percentage of approximately 25%
20 of cells containing B19 recombinant VP1 and spotted on 18-well
glass plates (nutacon, cat.no. 10-404) with a concentration of
approximately 500 cells/well (5 µl) and after drying in the
air fixated in 100% acetone for 20 min at -20°C. Then the
cells were incubated with human sera (positive and negative
25 sera for B19-Ig) at different dilutions in VBS (veronal
buffered saline). These sera were pretreated with liver powder
for a reduction of possible background colouring. After
incubation for 1-2 h at 37°C the glass plates were washed in
PBS and dried in the air before incubation with a 1:40
30 dilution of FITC-labelled goat-anti-human-IgG (Kallestad,
cat.no.:104) in VBS with 1:500 "Evans Blue" (Hoffmann La
Roche). After washing in PBS and drying in the air
Tris/glycerol buffer (1g Tris in PBS, 80% glycerol, pH 9.9)
was added and analysis done under the UV-microscope. In B19-
35 IFA's with positive patient sera the fluorescent cells could

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be observed. The usefulness of the B19-IFA was tested in a
number of experiments as described hereinbelow:

a) "Double-blind" experiment.
5   30 patient sera were tested according to the procedure in 1:50
and 1:500 dilutions, without the "investigator" knowing which
of the sera were positive and which were negative. After
reading by several co-workers the results were compared with
the data from the RIA test (Cohen et al., 1983; J. Hyg. 91,
10   113-130): in the B19-IFA all 17 IgG-positive sera were read as
positive and all 13 IgG-negative sera as negative.

b) Titration.
Of a number of B19-antibody positive sera (positive according
15   to the test results of the RIA, performed with virus isolated
from serum as antigen, Cohen et al., 1983; J. Hyg. 91, 113-
130) an IFA-titre was determined. Results are summarized in
Table 1. The data reveal that this B19-IFA yields results
corresponding to the RIA data: sera with high positive values
20   in the RIA also have high titres in the B19-IFA and sera with
low positive values in the RIA yield lower titres in the B19-
IFA.

15

Table 1:   Comparison between RIA and IFA for the assay of B19
antibodies

IgG RIA (a.u.)	IFA (titre)	IgM RIA (a.u.)	IFA (titre)
100	8 192	>100	1 280
100	>16 384	88	1 280
91	>16 384	50	1 280
70	8 192	31	2 560
55	8 192	25	1 280
40	8 192	16	640
25	8 192	16	320
15.5	4 096	8.3	40
11	4 096	5.3	<10
8.4	1 024	5	80
7.6	512	4.4	10
4.2	512	4.1	40
4.0	512	3.1	10
2.5	<32	3.4/2.9	<10
1.8	128	3.3	160
1.2	<32	2.3/1.9	<10
<1	<32	1.6	<10
<1	<32	<1	<10
<1	<32	<1	<10

a.u. = arbitrary units; <1 = negative value RIA; <32 =
negative value IFA at lowest dilution of IgG; <10 = negative
value IFA at lowest dilution of IGM.

c) Screening of donors.
100 randomly selected blood donors were screened for the
presence of B19-specific IgG antibodies and the results showed
that in this B19-IFA 76% of the donors were positive, which
corresponds very well with the data as described for the human
parvovirus B19 for this age-group.

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Example 2: Expression of parvovirus B19 VP2.

Subcloning of VP2 into pUC7.

5   Cloning of the VP2 of parvovirus B19 started from the B19 DNA
     that has been cloned into pUC19 according to the procedure as
     described in Example 1 under points 1 and 2. A 1.8 kb
     fragment, coding for VP2, was cut from the pUC19 construct
     (pUC19-B19VP1) with HpaII (bp3083) and EcoRI, isolated from
10   agarose gel, filled in with "Klenow large fragment DNA
     polymerase" and ligated with pUC7 plasmid cut with HincII. The
     new pUC7 construct (pUC7-B19VP2) was then propagated in E.coli
     JM101. Bacterium colonies were then tested for the presence of
     a B19-insertion by means of restriction enzyme analysis and
15   hybridization with a B19-specific DNA probe (Salimans et al.,
     1989; J. Virol. Meth. 23, 19-28). Further, the same procedure
     was followed as described for VP1 in Example 1 under points
     4-7 to generate recombinant baculovirus with the DNA coding
     for VP2 of the human parvovirus and to produce VP2 in the
20   insect cells (AcB19VP2L).

     Example 3: Expression of parvovirus B19 VP1 and VP2 using
     double infection of insect cells

25   Two days after infection with the recombinant viruses
     (AcB19VP1L and AcB19VP2L, m.o.i. 5) insect cells (10^6 Sf-cells
     in 35mm Petri-dish) were cultured for 4 h at 27°C in
     methionine-free "Grace" medium to which 100 µCi ^{35}S-methionine
     had been added, to determine the *de novo* synthesis of
30   proteins. After the supernatant was discarded, the cells were
     placed in PBS (phosphate buffered saline). Both supernatant
     and cell fraction were placed in lysing buffer, boiled for 5
     min and analyzed on a 10% SDS-polyacrylamide gel. The same
     experiments were performed with uninfected and wild type
35   baculovirus-infected cells. Autoradiography (not shown)
     revealed that the polyhedrin band (30kd), which dominated

strongly in the wild type infection, disappeared in the cells
infected with the recombinant viruses and that proteins of a
size of the VP1 (84 kD) and the VP2 (58 kD) of B19 were
synthesized. The results were confirmed by SDS-PAGE analysis
5   as described in Example 1 under point 9.

    Example 4: Production and purification of VP2 capsid protein
    of human parvovirus strain B19

10 Sf-cells were infected with the VP2-recombinant baculovirus
    (m.o.i. 20). 72 h after the initiation of the infection the
    cells were washed in PBS, harvested and then sonicated in PBS.
    Cell debris was removed by means of centrifugation (10 min,
    1,000 x g), after which the supernatant was placed on a 40%
15 sucrose cushion and centrifugation was performed for 2.5 h at
    100,000 x g. The pellet was resuspended in PBS and then
    separated on a linear sucrose gradient (15-30%, w/w). After
    centrifugation for 2.5 h at 110,000 x g an opalescent band was
    observed. Material from this band was examined by electron
20 microscopy. Particles were found that showed great similarity
    to the parvovirus capsid as to diameter (approximately 21 nm)
    and morphology. After analysis of the sucrose gradient
    fraction by means of SDS-PAGE and silver staining a protein
    was found of the size of VP2 (58K). By means of radio-immuno-
25 precipitation and Western blot analysis with a human serum,
    specific for parvovirus B19, it was demonstrated that the VP2
    protein is indeed involved here. The fact that after silver
    staining no other proteins were detected points to a high
    degree of purity. The amount of protein in the gradient
30 fractions enriched for the particles was determined by means
    of the Bradford procedure. On the basis of the data obtained
    the number of particles per infected insect cell was estimated
    at at least 10^5, it being assumed that each particle is made
    up of 60 molecules. To determine whether the purified VP2
35 particles can be used for setting up a diagnostic test, an
    ELISA was performed with 8 B19-positive and 3 B19-negative

human sera. In Table 2 the results are compared with those obtained for purified recombinant VP1 protein. All B19-positive sera recognized the VP2 capsids. Moreover, for seven of the eight sera the reaction with the VP2 capsids proved
5 more strongly positive than that with the purified VP1 protein.

Table 2: ELISA for demonstrating parvovirus-specific
10 antibodies in 11 human sera with recombinant VP1 and VP2 protein as antigens

Extinction*	number of sera	
	purified VP1	VP2 particles
<0.5	3	3
0.5-1.0	2	0
1.0-2.0	5	0
>2.0	1	8

* Extinction <0.5: negative; >0.5: positive
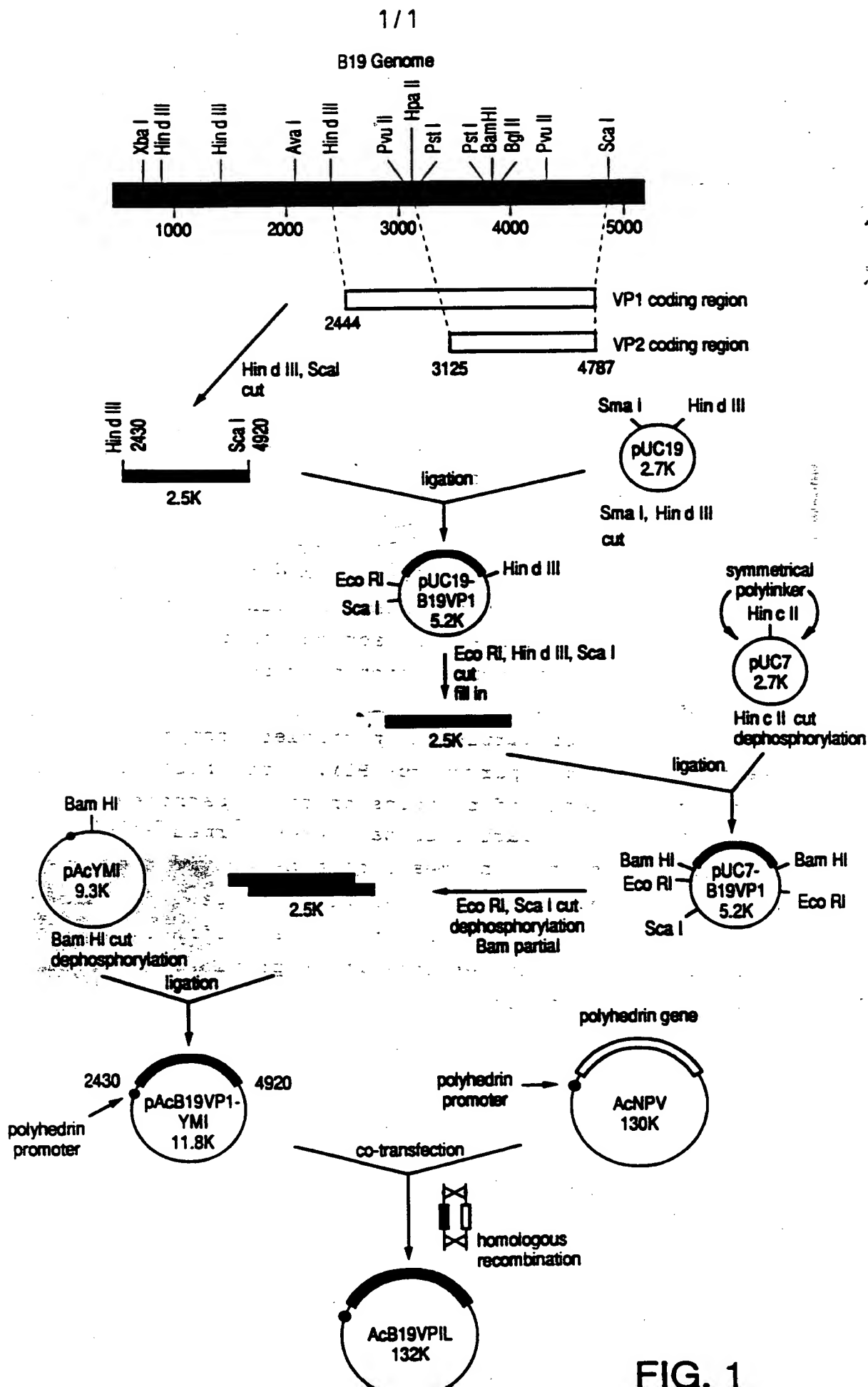
FIG. 1